Oral inflammatory diseases and systemic inflammation: role of the macrophage

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Inflammation is a complex reaction to injurious agents and includes vascular responses, migration, and activation of leukocytes. Inflammation starts with an acute reaction, which evolves into a chronic phase if allowed to persist unresolved. Acute inflammation is a rapid process characterized by fluid exudation and emigration of leukocytes, primarily neutrophils, whereas chronic inflammation extends over a longer time and is associated with lymphocyte and macrophage infiltration, blood vessel proliferation, and fibrosis. Inflammation is terminated when the invader is eliminated, and the secreted mediators are removed; however, many factors modify the course and morphologic appearance as well as the termination pattern and duration of inflammation. Chronic inflammatory illnesses such as diabetes, arthritis, and heart disease are now seen as problems that might have an impact on the periodontium. Reciprocal effects of periodontal diseases are potential factors modifying severity in the progression of systemic inflammatory diseases. Macrophages are key cells for the inflammatory processes as regulators directing inflammation to chronic pathological changes or resolution with no damage or scar tissue formation. As such, macrophages are involved in a remarkably diverse array of homeostatic processes of vital importance to the host. In addition to their critical role in immunity, macrophages are also widely recognized as ubiquitous mediators of cellular turnover and maintenance of extracellular matrix homeostasis. In this review, our objective is to identify macrophage-mediated events central to the inflammatory basis of chronic diseases, with an emphasis on how control of macrophage function can be used to prevent or treat harmful outcomes linked to uncontrolled inflammation.

Keywords: innate immune system, macrophage, oral disease, inflammation, resolution

INTRODUCTION

Inflammation is the physiological response of the body to injury. The inflammatory response can be either acute and of short duration or chronic, which does not resolve and leads to pathology. The major function of innate immune cells most studied during the inflammatory process is the identification and recognition of the injurious and/or foreign substances promoting the defense response. Less acknowledged roles played by the innate immune cells involve the resolution pathways and wound healing, both of which include repair and regeneration of lost or damaged tissues. These are now recognized as highly regulated, active processes rather than passive events (Van Dyke, 2008). Macrophages are actively involved in all phases of inflammation and their role as effector and regulatory cells is now widely recognized. Another interesting and important feature of macrophages is their high level of specialization and tissue specificity. While all tissue-bound macrophages differentiate from circulating monocytes, they acquire distinct characteristics and functions locally due to their response profiles. One of the major factors for this diversity is the complexity of microbial load as well as tissue architecture. Thus, it is no surprise that some of the most sophisticated interactions between the host and parasites also dictate the most evolved phenotypic characteristics of the macrophage. Some examples of this specificity and complexity of macrophage phenotype and function are the Kupffer cells of the liver and macrophages of the lung alveoli where the cells, while similar in appearance, are involved in distinct responses against different pathogens as well as non-pathogenic stimuli.

The oral cavity is one of the most ecologically complex microenvironments in the human body where interactions between the host and microbes define health and disease (Gemmell et al., 1997). The teeth are the only functional hard tissues extending from inside to outside of the human body crossing a series of other hard (i.e., bone) and soft (i.e., connective tissue and epithelia) tissues surrounded by a tight biofilm formed by the richest collection of bacteria outside the colon. Such architecture creates several zones, which work in concert during the inflammatory responses in the mouth. Regulation of immune-inflammatory mechanisms in oral disease is governed in part by patient susceptibility and environmental factors (Seymour, 1991; Seymour and Gemmell, 2001; Uitto et al., 2003). In particular, oral macrophages address these complex requirements for mounting a successful inflammatory response as the cell type at the center of many processes including signaling to resolution of inflammation, healing, and regeneration. In this review, within the context of pathogenic mechanisms, possible clinical outcomes will be
discussed in relation to the inflammatory–immunological changes throughout the disease process. Since most inflammatory diseases of the oral cavity involve the tissues of periodontium, the pathological changes in the periodontal structure will be used as a model to assess the role of the macrophages in oral inflammation and its resolution.

**ACTIVATION OF ORAL INFLAMMATION AND THE ROLE OF MACROPHAGES**

Typically, there are two common diseases affecting the oral tissues and the health of the supporting structures of a tooth. In the case of *gingivitis*, inflammation is limited to the soft tissues, epithelium, and connective tissue; or in the case of *periodontitis*, inflammatory processes extend to the supporting tissues including the alveolar bone (Page and Schroeder, 1976). In both forms of periodontal inflammation, the pathological consequences are associated with the accumulation of bacteria at the tooth surface leading to a host response generating inflammatory cell infiltration (Socransky and Haffajee, 2005). Since the soft and hard tissues of the oral cavity are part of the same functional and physiological organ, separating the host response to several components is artificial and does not acknowledge the dynamic relationship between the cells, bacteria, and extracellular structures. Likewise, while practical and instructive, the supposition of a linear shift in lesions from acute to chronic is not clear. Recent discoveries defining the pathways of resolution in the inflammatory processes challenge the concepts of compartmentalization and linearity in acute and chronic responses (Serhan, 2010; Pruss et al., 2011). Nevertheless, this is the prevailing paradigm, since the tools for analyses of the events at multiple levels are just being incorporated into oral research (Singh et al., 2011; Hasturk et al., 2012). Based on the preliminary results of studies that use high-throughput measurements to generate a systems-biology approach, the complex nature of host–bacteria interactions in a highly complex environment of the oral cavity is being redefined (Bakthavatchalu et al., 2011; Mishima and Sharma, 2011; Singh et al., 2011). To this end, novel approaches have revealed the orchestrated coupling of activation and resolution phases as well as tissue healing.

Macrophages are central to the coordinated resolution of inflammation and return to tissue homeostasis (Zadeh et al., 1999). During the first step of the inflammatory process directed against microorganisms, bacteria, and their virulence factors (e.g., capsule, lipopolysaccharide, fimbria) trigger receptor-mediated production of cytokines by epithelial cells with simultaneous release of neuropeptides, which cause vasodilatation of local blood vessels. Generation of chemotactic agents (chemokines) at this stage results in attraction of the first line of defense, the neutrophil, which will leave the vessels and migrate to the site of microbial invasion. This step is critical and plays a pivotal role in generation of an effective defense system. Neutrophils are followed by the macrophages. This is the step usually where clinical signs of oral inflammation including bleeding, swelling, and redness of the gingiva are detectable. The inflection can either be confined and cleared by the function of neutrophils and macrophages at this early stage, or expand to include the other cells and structures (Page and Schroeder, 1976). Being myeloid cells of hematopoietic origin (Medzhitov and Janeway, 1997; Janeway and Medzhitov, 2002) the overall role of the macrophages is to limit the pathological changes to the soft tissues or elevate the inflammatory response to the next level. Major functions of macrophages include elimination of invading bacteria, recruitment of other cells to the site of infection, clearance of the excess neutrophils, production of cytokines and chemokines, and activation of the lymphocyte-mediated adaptive immune response. The net outcome of these functions can be either complete resolution with healing, limiting the infection with resultant fibrosis and healing with scar tissue formation, or a failure to clear the infection with establishment of a chronic inflammatory lesion.

In the case that the inflammatory process is prolonged and becomes chronic, destruction of soft and hard tissues including the alveolar bone is observed due to direct tissue destruction mediated by inflammation (McCauley and Nohutcu, 2002; Hasturk et al., 2006; Taubman et al., 2007; Graves, 2008; Li et al., 2011). Macrophages together with neutrophils are responsible of phagocytosis and digestion of microorganisms and foreign substances through surface receptors that recognize and bind certain surface molecules of bacteria such as the lipopolysaccharides (LPS; Medzhitov and Janeway, 1997). These receptors are the key components for distinguishing between the host and the invader and defined as pathogen recognition receptors called toll-like receptors (TLR; Anderson, 2000), which mediate the elimination of the pathogenic microbes through phagocytosis and killing (Wingrove et al., 1992). TLRs regulate apoptosis, inflammation, and immune responses (Anderson, 2000). Evidence supporting a role for TLR-mediated recognition of macrophage function in resolution of inflammation is accumulating providing strong support indicating that this receptor–ligand interaction is key to the homeostatic restoration of the host defense (Duffield et al., 2006; Schif-Zuck et al., 2011). Recently, a group of nucleotide-binding oligomerization domain proteins (NODs) have been described as potential regulators of apoptotic events and nuclear factor κB (NF-κB) activation within the context of pathogen recognition and the inflammatory responses (Inohara and Nunez, 2003). While it is not clear how NODs are involved in oral inflammatory diseases, evidence suggests that they are expressed in gingival cells and may play role in promotion of oral inflammation (Uehara and Takada, 2007; Tang et al., 2011).

The TLR family is the best-characterized class of pathogen recognition receptors. TLRs are unique receptors that recognize molecules, broadly shared by microorganisms, but are distinguishable from the host molecules, referred to as “pathogen-associated molecular patterns (PAMP).” TLRs detect multiple PAMPs, including LPS, bacterial lipoproteins and lipoteichoic acids, flagellin, CpG DNA of bacteria and viruses, double-stranded RNA, and single-stranded viral RNA (Iwasaki and Medzhitov, 2004). To date, 11 different TLRs have been identified (Liu et al., 2000; Takeda et al., 2003; Krutzik and Modlin, 2004; Quesniaux et al., 2004). When TLRs bind to antigens, series of intracellular events are initiated and the process leads to the production of cytokines, chemokines, and antimicrobial peptides (Donati et al., 2009). The binding can be through four different adapters. Each adapter has the potential of producing various cytokines stimulating NF-κB pathway in the nucleus of the cell. Known adapter proteins of TLRs are MyD88, toll–interleukin-1 receptor domain...
containing adapter protein (TIRAP), toll–interleukin-1 receptor domain containing adapter-inducing interferon-β (TRIF) and TRIF-related adapter molecule (TRAM). TLRs also utilize interleukin-1 receptor-associated kinase (IRAK), and TNF receptor-activated factor 6 (TRAF6; Jiang et al., 2000). Different TLRs induce different responses; for example, in dendritic cells, the interaction of TLR 4 and LPS results in the production of pro-inflammatory cytokines such as interleukin-12. TLR-2 and TLR 4 have been shown to be expressed in oral tissue cells. The same TLR can trigger different responses through different intracellular adapter proteins (Alexopoulou et al., 2001; Kaisho and Akira, 2002; Cook et al., 2004; Krutzik and Modlin, 2004; Watters et al., 2007). TLRs 1, 2, 4, 5, and 6 specialize in the recognition of mainly bacterial products that are unique to bacteria and not made by the host. This gives them the specificity to differ the invader from the host (Iwasaki and Medzhitov, 2004). Recognition by the TLR pathway is a crucial phase in inflammation. After recognition, many cytokines are released from various cell types including the macrophages through the NFκB pathway (Uehara and Takada, 2007). After TLR4 activation, MyD88 is recruited to TLR4 through respective Toll/IL-1 receptor (TIR)–TIR interactions (Medzhitov, 1998; Muzio et al., 1998; Raschi et al., 2003). MyD88 also contains a death domain (DD), a highly conserved protein-binding domain that facilitates its interaction with another DD-containing signaling molecule, IRAK (Cao et al., 1996). IRAK subsequently undergoes phosphorylation and dissociates from MyD88, interacts with TRAF6, and thereby activates several downstream kinases (Cao et al., 1996; Yamin and Miller, 1997; Aderem and Ulevitch, 2000; Jiang et al., 2000; Swantek et al., 2000; Raschi et al., 2003). Following LPS stimulation, two signaling pathways have been described, the MyD88-dependent and -independent pathways (Akira et al., 2000; Kawai et al., 2001; Sato et al., 2002; Yamamoto et al., 2003). Endotoxin activation of the MyD88-dependent pathway results in rapid NF-κB activation and release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and IL-1β. Endotoxin activation of the MyD88-independent pathway results in rapid activation of interferon regulatory factor 3 (IRF3) leading to beta interferon (IFN-β) release with delayed NF-κB activation (Akira et al., 2000; Kawai et al., 2001; Hoebe et al., 2003). The TLR proteins possess leucine-rich extracellular repeats that recognize the LPS binding protein (LBP)–CD14 complex (Poltorak et al., 2000). The TLR intracellular domain resembles the IL-1β receptor, hence the term TIR homology domain (Medzhitov et al., 1997; Chaudhary et al., 1998; Rock et al., 1998). The TIR domain in the cytoplasmic portion of the molecule is considered essential for triggering activation of mitogen-activated protein kinases (MAPKs) and the transcription factor NF-κB (Means et al., 2000; Akira et al., 2001; Sato et al., 2002). While CD14 is the major LBP on the surface of mononuclear phagocytes, CD14 is not capable of transducing signals across the membrane. A receptor complex comprised of CD14, TLR-2, TLR-4, and accessory proteins (MD-2) is necessary for receptor function as well as various kinases, including the three classes of MAPK: extracellular signal-regulated kinase (ERK) 1 and ERK2 (Weinstein et al., 1992), p38 MAPK (Han et al., 1994), and c-Jun N-terminal kinases (JNK; Hambleton et al., 1996). Numerous inflammatory cytokines and mediators are expressed in LPS stimulated macrophages through activation of transcription factors including NF-κB and activator protein-1 (Fujihara et al., 1993; Muroi et al., 1993; Guha and Mackman, 2001). LPS recognition is initiated by LBP, a serum glycoprotein, that first binds to the lipid A moiety of LPS (Schumann et al., 1990; Wright et al., 1990; Gegner et al., 1995). The LPS–LBP complex is then recognized by CD14 (Schumann et al., 1990; Ulevitch and Tobias, 1995; Haziot et al., 1996). Mice with a targeted deletion of the gene encoding CD14 are hyporesponsive to LPS and resistant to the lethal effects of LPS (Haziot et al., 1996). However, mice lacking CD14 are still able to respond to high concentrations of LPS (Wurzel et al., 1997). CD14 is a glycosylphosphatidylinositol-anchored (GPI-anchored) molecule which lacks a cytoplasmic signaling domain, making it incapable of downstream signaling (Haziot et al., 1988). It is not fully clear if the TLR-mediated pathways are directly involved during the oral inflammatory responses including resolution.

We have previously identified moesin as a participant in LPS binding and signal transduction (Tohme et al., 1999). Many physiological and pathophysiological conditions are attributable in part to cytoskeletal regulation of cellular responses to signals. Moesin is an ERM (ezrin, radixin, and moesin) family member and was identified as part of a protein cluster. Moesin was found to be necessary for the detection of LPS, and homozygous moesin knockout mice exhibited a threefold reduction in neutrophil infiltration into LPS injected sites when compared to wild type controls (Amar et al., 2001). Anti-moesin antibody inhibited the release of TNF-α by LPS stimulated monocytes (Tohme et al., 1999), and moesin was also found to be expressed on the surface of differentiated THP-1 cells and primary peripheral blood monocytes. LPS stimulation increased the surface expression of moesin as well as its total protein levels when analyzed by FACS and Western blotting, respectively. Furthermore, moesin was found to co-immunoprecipitate with TLR4 after LPS stimulation (Iontcheva et al., 2004). In moesin mRNA knockdown experiments using antisense mRNA, THP-1 cells no longer responded to LPS (Iontcheva et al., 2004), suggesting a role for moesin in LPS signaling. Using differentiated THP-1 cells, co-immunoprecipitation experiments revealed that moesin and CD14 were associated in the cell membrane in both resting and LPS stimulated cells. TLR4 and MD-2 became associated with moesin and CD14 only after LPS stimulation. These experiments also demonstrated that there was a direct binding between moesin and LPS. Moesin was phosphorylated and mRNA levels of moesin increased significantly after LPS stimulation. During the TLR4-mediated response to LPS, moesin stimulates the NF-κB, p38, and p44/42 MAPK activation (Iontcheva et al., 2004; Zawawi et al., 2010). Figure 1 shows a model for recognition of LPS involving the dynamic association of multiple molecules, including moesin, forming a cluster that functions as the LPS receptor and an important role in the macrophage-mediated innate immune response and TLR4-mediated pattern recognition in oral inflammatory diseases.

Cytokines and other products of macrophages can also modulate the action, differentiation, and survival of cells outside the immune system, such as the nervous system. The interaction between macrophages and the nervous system relies on the receptor-sensitizing characteristics of cytokines (Opree and Kress, 2000) linked to the discovery of protease-activated receptors...
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FIGURE 1 | Lipopolysaccharides (LPS) recognition and signaling in macrophages. CD14 and moesin are expressed on the cell membrane in macrophages. LPS stimulation results in phosphorylation of moesin, binding to the TLR4 and MD-2 activating the MyD88. Signaling through this mechanism leads to the production of pro-inflammatory cytokines (Zawawi et al., 2010).

PARs. Research into the functionality of these receptors has shown that PAR-2 has a particularly important role in disease states associated with chronic inflammation (Vergnolle, 1999). Identification of neuropeptide receptors on immune cells indicates a communication between the immune and neurological systems that possibly results in the modulation of inflammatory response through G-protein-coupled receptors located on the cell membranes or the vanilloid receptor-1 (also named TRPV1), which is shown to be up-regulated in inflammatory bowel disease. These findings suggest a possible role for this receptor in chronic inflammation (McGillis et al., 1991; Yangou et al., 2001; Tracey, 2002; Lundy and Linden, 2004). Cytokines have been shown to regulate substance P expression and response to LPS (Kessler and Freidin, 1993; Hua et al., 1996). Substance P limits the production of TGF-β by macrophages and induces synthesis of IL-6 (Lieb et al., 1996; Marriott and Bost, 1998). Macrophages can produce substance P when activated with LPS in vitro (Lambrecht et al., 1999). The precise mechanism through which these receptor-mediated events might regulate the macrophage response in the oral cavity is not clear; future research is needed to understand their role.

ROLE OF MACROPHAGES IN GINGIVAL INFLAMMATION AND BONE RESORPTION

Macrophages efficiently ingest particulate antigen, express MHC class II molecules and have co-stimulatory activity on T cells. Macrophages can be phenotypically polarized by the microenvironment. The classically activated macrophages (M1) are activated by IFN-γ and LPS, and alternatively activated macrophages (M2) produced in response to IL-4 or IL-13 (Martinez et al., 2009). M2 macrophages have been shown to play a role in resolution of inflammation with a reduced capacity to produce cytokines (Bhatavadekar and Williams, 2009). Cytokine and chemokine production by macrophages is a key step in immune response and the inflammation process. Cytokines interact between each other, amplifying signaling, modulate cell surface receptors, and perform synergistic or antagonistic interactions on cell function (Balkwill and Burke, 1989). It is not only the presence of one cytokine that regulates the response, but the concentration of the same mediator can also affect the outcome of a response (Gemmell et al., 1997). Their secretion is dependent on the NF-κB in the nucleus of many immune system cells (Baldwin, 1996; Kaneda and Yoshimura, 2002). In addition to macrophages, cytokines can be produced by both resident cells such as epithelial cells, fibroblasts and other phagocytes such as neutrophils in the periodontal tissues (Ara et al., 2009). After microbial recognition, cytokines in innate response such as TNF-α, IL-1, and IL-6 are the first to start communication in disease pathogenesis (Garlet, 2010). IL-1β and IL-6 are the signature innate cytokines and have been characteristically associated with inflammatory cell migration, highly produced by the macrophages and involved in osteoclastogenesis processes (Graves et al., 2008; Fonseca et al., 2009). TNF-α is a multi-role cytokine, that has many functions from cell migration to tissue destruction. It induces the up-regulation of adhesion molecules, stimulates the production of chemokines, and is involved in cell migration to infected and inflamed sites (Peschon et al., 1998; Dinarello, 2000; Wajant et al., 2003; Kindle et al., 2006). TNF-α up-regulates the production of other signature pro-inflammatory innate immunity cytokines, such as IL-1β and IL-6 (Okada et al., 1997; Dinarello, 2000; Wajant et al., 2003; Kwan Tat et al., 2004; Garlet et al., 2007; Graves et al., 2008; Musacchio et al., 2009). TNF-α is also correlated with extracellular matrix (ECM) degradation and bone resorption through its positive correlation with matrix metalloproteinases (MMPs) and RANKL expression (Graves and Cochran, 2003; Garlet et al., 2004; Graves et al., 2008). Experimental periodontitis in TNF-α p55 receptor deficient mice was characterized by a significant decrease in MMPs and RANKL expression (Garlet et al., 2007). Thus, in addition to direct actions in bone resorption, macrophage-derived cytokines also interfere...
with the coupled bone formation process (Behl et al., 2008). IL-13 is another potent modulator of human monocyte/macrophage function. Monocyte/macrophage cell surface markers, MHC class II and several integrin molecules are up-regulated by IL-13 (de Waal Malefyt et al., 1993). The monocyte/macrophage related production of the cytokines IL-1α, IL-1β, IL-6, IL-8, and TNF-α is also inhibited by IL-13. On the other hand, IL-1 receptor antagonist secretion is enhanced (de Waal Malefyt et al., 1993; Zurawski and de Vries, 1994). Therefore, IL-13, along with IL-4 and IL-10, would appear to have potential anti-inflammatory activity (Zurawski and de Vries, 1994).

In addition to their trafficking role, chemokines provide messages leading to other biological processes, such as angiogenesis, cell proliferation, apoptosis, tumor metastasis, and host defense (Rossi and Zlotnik, 2000; Zlotnik and Yoshie, 2000; Moser et al., 2004; Rot and von Andrian, 2004; Esche et al., 2005). Chemokines are classified into four subfamilies according to the configuration of cysteine residues near the N-terminus. Chemokines engage their receptors. This binding initiates integrin-dependent adhesion, as well as the binding and detachment of cells from their substrate. Chemokines target all types of leukocytes of the innate immune system, as well as lymphocytes of the adaptive immune system (Terricabras et al., 2004). IL-8/CXCL8 is the first cytokine identified to have chemotactic activity. It can be produced by macrophages as well as fibroblasts, epithelial cells, and endothelial cells (Takashiba et al., 1992; Takigawa et al., 1994; Yumoto et al., 1999). IL-8 is a neutrophil chemoattractant. It is detectable in healthy and diseased periodontal tissues and has been associated with subclinical inflammation (Yoshimura et al., 1987; Payne et al., 1993; Mathur et al., 1996). It has direct action on osteoclast differentiation and activity by signaling through the specific receptor, CXCR1 (Bendre et al., 2003). Another crucial chemokine for macrophage function is MCP-1/CCL2, which mediates the recruitment of monocytes/macrophages (Hanzawa et al., 1993; Okamoto et al., 2004). Together with RANTES/CCL5, MCP-1α/CCL3 may also be involved in the migration of macrophages to oral tissues (Gemmell et al., 2001; Kabashima et al., 2002). CXCR3 and its ligand IP-10/CXCL10 are also expressed in diseased periodontal tissues and associated with higher levels of IFN-γ during the inflammation process (Kabashima et al., 2002; Garlet et al., 2003). CXCR4 is found expressed at higher levels in chronic periodontitis and it is associated with higher levels of IL-4 and IL-10 messages in the periodontium (Garlet et al., 2003, 2004).

In addition to recruitment of cells, chemokines are crucial in guiding adaptive immunity cells with a role in bone metabolism. MDC/CCL22, TARC/CCL17, and 1-309/CCL1 have been shown to attract Th2 and Treg cells via binding their CXCR4 and CXCR8 receptors (D’Ambrosio et al., 1998; Sallusto et al., 1998; Gu et al., 2000). Chemokines have been recognized as essential signals for the trafficking of osteoblast and osteoclast precursors, and consequently as potential modulators of bone homeostasis (Bendre et al., 2003; Wright et al., 2005). Chemokines are capable of regulating bone metabolism via CCR1, CCR2, CXCR3, and CXCR4 receptors expressed on osteoclast precursors, mature osteoclasts, and osteoblasts. These receptors have the ability to bind many different chemokines such as SDF-1/CXCL12, MIP-1α/CCL3, RANTES/CCL5, MIP-1γ/CCL9, MCP-1β/CCL2, MCP-3/CCL7, MIG/CXCL9, and CKβ8/CCL23 (Votta et al., 2000; Lean et al., 2002; Okamatsu et al., 2004; Yu et al., 2004; Kwak et al., 2005; Wright et al., 2005; Kim et al., 2006a,b; Yang et al., 2006). IP-10/CXCL10 induces osteoblast proliferation through receptor CCR3 (Grassi et al., 2003; Lisignoli et al., 2004), while SDF-1α/CXCL12 and BCA-1/CXCL13 induce both proliferation and collagen type I mRNA expression in osteoblasts through receptors CCR4 and CCR5 (Lisignoli et al., 2006). In addition to its role in osteoclastogenesis, chemokines also affect osteoclast functions. It has been reported that SDF-1α/CXCL12 increases MMP-9 activity in human osteoclasts, resulting in increased bone resorption activity (Grassi et al., 2004). There is evidence that RANTES/CCL5 can also act on osteoblasts, resulting in chemotaxis and promoting cell survival (Yano et al., 2005). RANKL also induces the production of MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5, and MIG/CXCL9 by osteoclasts, suggesting a coupling role, which could contribute to bone resorption (Kim et al., 2006a). Taken together, these studies suggest that macrophage-produced chemokines can effectively contribute to the bone remodeling process by driving osteoblast migration and activation during periodontal wound healing.

An important key mediator of macrophage function is the prostaglandins, which are derived from hydrolysis of membrane phospholipids. Phospholipase A2, cleaves arachidonic acid, a precursor of small lipids known as eicosanoids, from membrane phospholipids. Eicosanoids generally act as inflammatory agents (Lewis, 1990). Arachidonic acid is metabolized via two enzymatic pathways. The first is the action of lipoxigenases that results in the formation of hydroxyeicosatetraenoic acids (HETE) and leukotrienes (LT). Alternatively, cyclooxygenases (COX) catalyze the conversion of arachidonic acid into prostaglandins, prostacyclins, and thromboxanes. Prostaglandins have 10 sub-classes, of which D, E, F, G, H, and I are the most important (Gemmell et al., 1997). Inflamed oral tissues synthesize significant amounts of prostaglandins (Mendieta et al., 1985). Prostaglandin E2 is the most potent stimulator of alveolar bone resorption (Goodson et al., 1974; Dietrich et al., 1975). Within oral lesions, prostaglandin E2 is mainly localized within macrophage-like cells and secreted when stimulated with bacteria LPS (Loning et al., 1980). Periodontal ligament cells also produce prostaglandin E2 even at rest. This secretion is enhanced by IL-1β, TNF-α, and parathyroid hormone (Richards and Rutherford, 1988; Saito et al., 1990a,b). Prostaglandin E2 has a bifasic action on cells; in high doses, it decreases IgG levels but in low doses has the potential to increase them. When combined with IL-4, low doses of prostaglandin E2 induce a synergistic rise in IgG production, suggesting an immune-regulatory role for prostaglandin E2 (Harrell and Stein, 1995).

Disruption of the balance between osteoblast and osteoclast activities by bacterial products and inflammatory cytokines constitute the main underlying causes of inflammation-induced bone loss (Liu et al., 2000). It is shown that LPS of bacteria either directly or through its action on the macrophages is capable of stimulating bone resorption when added to osteoclast precursor cultures containing osteoblasts and/or stromal cells (Ino and Hopps, 1984). In addition to this, TLR and inflammation-induced osteoclastogenesis pathway is the most common pathway related to bone loss (The American Academy of Periodontology Academy
Inflammation-induced and macrophage-mediated bone loss in oral infection involves complex inflammatory signals and cytokine networks regulating osteoclastogenesis, such as RANKL, interleukin-1, interleukin-6, tumor necrosis factor-α, and prostaglandin E2 have been reported to be significantly associated with this type of tissue destruction (Henderson et al., 2003). Before the discovery of receptor activator of NFκB (RANK), its ligand (RANKL), and its antagonist osteoprotegerin (OPG), the development and formation of osteoclasts were attributed to factors produced by osteoblasts and bone marrow stromal cells (Rodan and Martin, 1981; Martin and Sims, 2005). It is now clear that RANKL, RANK, OPG are the key regulators of bone remodeling, directly involved in the differentiation, activation, and survival of osteoclasts and osteoclast precursors (Anderson et al., 1997; Lacey et al., 1998; Yasuda et al., 1998). RANKL is expressed by osteoblasts, stromal cells, chondrocytes, and other mesenchymal cells. Activated T and B cells can also express RANKL (Thehill et al., 2002; Mahamed et al., 2005; Kawai et al., 2006). RANK is expressed by osteoclast progenitors, mature osteoclasts, chondrocytes, monocytes/macrophages, and dendritic cells (Anderson et al., 1997; Hsu et al., 1999). Their decoy receptor OPG is known to be expressed by periodontal tissue cells like fibroblasts and periodontal ligament cells (Liu et al., 2000). Blocking RANKL activity with OPG significantly inhibits bone loss in rheumatoid arthritis, osteoporosis, cancer-related bone metastasis, and diabetes associated alveolar bone destruction (Mizuno et al., 1998; Kong et al., 1999; Honore et al., 2000; Brown et al., 2004; Holbauer and Schoppet, 2004; Mahamed et al., 2005), confirming the critical role of the RANKL, RANK, OPG triad in osteoclastogenesis. Osteoclastogenesis via RANKL, RANKL pathway depends on Macrophage-Colony Stimulating Factor (M-CSF; Tanaka et al., 1993; MacDonald et al., 2005). Pathogens, stress, or pathology influence the production of M-CSF via pro-inflammatory cytokines and have a significant role on the subsequent osteoclast activity where TLR-2 activation up-regulates the expression of M-CSF (Song et al., 2009). LPS from different pathogens can stimulate bone resorption in vitro and in animal models as in primary mouse calvarial osteoblasts, the activation of TLR-2 and TLR-6 by LPS causes enhanced expression of RANKL through a MyD88-dependent mechanism (Sato et al., 2004). In mouse calvarial osteoblasts, expression of TLR 4 and TLR-9 results in the activation of NFκB and related to that the increased secretion of TNF-α and M-CSF (Morse et al., 2008). LPS-induced interleukin-1 production through TLR pathway can up-regulate RANKL and inhibit osteoprotegerin expression by osteoblasts resulting in osteoclast formation in a prostaglandin E2-dependent manner. TLR-2 substantially decreases the response to LPS (Song et al., 2009). LPS directly, or via TLR pathway by stimulating different cell types is capable of inducing osteoclast development and activity. Thus, TLRs could influence the inflammatory response in the bone microenvironment, and may play a critical role in modulating inflammation-induced osteoclastogenesis and bone loss.

Another mechanism underlying macrophage involvement in oral tissue pathologies is the destruction of ECM. Collagenases, along with other MMPs, play an important role in this process. MMPs are a family of structurally related but genetically distinct enzymes that degrade ECM and basement membrane components. Twenty-three enzymes have been classified into collagenases, gelatinases, stromelysins, membrane-type MMPs, and other MMPs, mainly based on the substrate specificity and molecular structure. MMPs are involved in physiological processes such as tissue development, remodeling, and wound healing. MMP activity is controlled by changes in the delicate balance between the expression and synthesis of MMPs and their major endogenous inhibitors, tissue inhibitors of MMPs (TIMPs). It is clear that MMPs are up-regulated in periodontal as well as other types of oral inflammation (Ebert et al., 2005). MMP activation involves tissue and plasma proteinases and bacterial proteinases together with oxidative stress (Henry et al., 2002; Rot and von Andrian, 2004). It is now clear that a broad range of cell types present in the normal and diseased human periodontium such as gingival sulcular epithelial cells, fibroblasts and endothelial cells, monocytes/macrophages, neutrophils, and plasma cells has the ability to express distinct MMPs (Sorsa et al., 1995; Takagi et al., 1995; Kiili et al., 2002; Wahlgren et al., 2002).

Matrix metalloproteinases gene transcription is very low in the healthy periodontal tissue; their secretion is stimulated or down-regulated by various cytokines. The main stimulatory cytokines for MMPs are TNF-α, IL-1, and IL-6. Activated MMPs are capable of activating other MMPs (Visse and Nagase, 2003). There is a close interaction between MMP activation and cytokine function. IL-1β and TNF-α can stimulate MMP-3, MMP-8, and MMP-9 secretions from gingival fibroblasts and MMP-13 in osteoblasts. TGF-β1 suppresses MMP-1, MMP-3, and MMP-8 gene transcription but induces MMP-2 and MMP-13 in keratinocytes (Birkedal-Hansen, 1993; Kahari and Saarialho-Kere, 1999; Könttinen et al., 1999). MMP-1 (collagenase-1) has a wide range of substrates. It can digest interstitial collagen, ECM components, and soluble non-matrix mediators (Sorsa et al., 2006). MMP-9 (gelatinase B) is a gelatinolytic enzyme degrading several ECM proteins, including basement membrane-type IV collagen (Lee et al., 1995; Sumer et al., 2010; Kantarci et al., 2011). MMP-9 is found to be expressed in epithelial cells; its production can be stimulated by several cytokines such as TNF-α, growth factors such as epidermal growth factor, and by some bacterial products such as LPS (Puttnins et al., 1996; Firth et al., 1997). Although MMP-1, MMP-8, and MMP-9 are the main enzymes that are involved in the ECM and base membrane breakdown, other MMPs and their tissue inhibitors (TIMPs) have also been linked to periodontal diseases. MMP-2 (gelatinase A) has been shown to be strongly expressed in inflamed pocket epithelium and to be important in epithelial cell migration (Makela et al., 1999). MMP-13 (collagenase-3) is expressed by the basal cells of the gingival pocket epithelium able to degrade collagens type I, III, and IV as well as fibronectin, tenasin, and some proteoglycans (Kahari and Saarialho-Kere, 1997; Knauper et al., 1997; Uitto et al., 1998). MMP-13 plays an important role in the ability of pocket epithelium to invade periodontal connective tissue. Some oral bacterial species, especially Fusobacterium nucleatum were found to induce MMP-13 (Uitto et al., 2003).

**RESOLUTION OF ORAL INFLAMMATION AND THE ROLE OF MACROPHAGES**

Oral inflammatory diseases can be considered as an adverse outcome of the protection efforts of the host against the invading...
pathogens. Inflammation should resolve in a timely manner to prevent tissue injury, and maintain health. The rapid and complete elimination of invading leukocytes from a lesion is the ideal outcome following an inflammatory event (Schwab et al., 2007). Inadequate resolution and failure to return tissue to homeostasis results in neutrophil-mediated destruction and chronic inflammation (Van Dyke and Serhan, 2003). If the host is unable to neutralize the pathogens, then acute inflammation would become chronic with consequences such as destruction of ECM and bone, scarring, and fibrosis (Van Dyke, 2008). Controlling the invasion of the neutrophils can impact the conversion of an acute gingivitis to chronic periodontitis. Scarring and fibrosis in periodontitis prevents the return to homeostasis (Van Dyke, 2007). When tissue injury is mild, necrotic cells will be replaced by new cells by regeneration process. If tissue damage is extensive, the process of healing is repair. When repair takes place, fibrin is not cleared rapidly and efficiently after the acute phase of inflammation and granulation tissue is formed from surrounding tissue compartments. Later phases of repair involve fibroblast-mediated collagen deposition, disappearance of vascular tissues and replacement of these areas by avascular and fibrotic scar tissue (Kumar et al., 2005). The efforts to control inflammation process has been mainly with the use of pharmacologic agents, which act as antagonists for some of the mediators of inflammation (Serhan et al., 2007). The resolution of inflammation previously thought to be a passive event, but greater understanding of the pathways and processes underlying resolution of inflammation has led to the recognition of an active progress where the activation of pro-resolving molecules are needed to neutralize and eliminate inflammatory leukocytes, and thereby prevent pathology (Van Dyke and Serhan, 2003; Van Dyke, 2007; Serhan et al., 2008). Restoration of tissue homeostasis is initiated following an acute inflammatory response that generates lipid mediators of inflammation (Van Dyke, 2008). Various lipid mediators such as eicosanoids, prostanooids, and prostacyclins are produced upon agonist stimulation of G-protein receptors on the cell membrane. Arachidonic acid (AA) plays key role in this process (Kantarci and Van Dyke, 2003) and is metabolized either by a cyclooxygenase (COX)-1 or COX-2-dependent pathway that results in the generation of prostanooids or a 5-lipoxygenase (5-LO)-dependent pathway that results in leukotriene (LT) production.

There is a high concentration of cells containing lipoxygenases, and corresponding pro-inflammatory products, a "class switch" may occur within neutrophils (Levy et al., 2001; Van Dyke, 2007). This class switch gives rise to the synthesis of pro-resolving molecules. One of the active resolution molecules is lipoxins, which are generated late in inflammation when a second lipoxygenase interacts with a lipoxygenase product generated earlier by a different cell (Serhan, 2004). These molecules are synthesized through a series of enzymatic reactions starting with the oxidation of AA by 15 LO through the process of transcellular biosynthesis, resulting in 15-S-hydroxy-(p)-eicosatetraenoic acid [15-S-H(p)ETE]. Accordingly, 15-S-H(p)ETE is further acted on by 5-LO to induce the synthesis of lipoxins, such as lipoxins A4 (LXA4) and B4 (LXB4; Kantarci and Van Dyke, 2003; Van Dyke and Serhan, 2003). The lipoxins produced act as agonists to stimulate the resolution of inflammation and promote the restoration of tissue homeostasis through a number of mechanisms. These include limiting PMN migration into sites of inflammation, activating monocytes without the generation of a superoxide anion, and stimulating the uptake of apoptotic PMN by macrophages (Serhan et al., 1993; Maddox and Serhan, 1996; Maddox et al., 1997). When the lipoxin pathway is activated and aspirin is present during this synthesis, acetylation of the COX-2 enzyme occurs to inhibit further production of prostanooids from AA metabolism. This alternative pathway will lead to the synthesis of 15-R-H(p)ETE transforming to 5(6)-epoxyeicosaetraene with the help of 5-LO activity. The next step is the synthesis of 15-epi-Lxs or aspirin-triggered lipoxins (ATLs) from 5(6)-epoxyeicosaetraene (Van Dyke and Serhan, 2003). 15-epi-LX is a form of native lipoxin and possesses potent pro-resolving properties (Serhan et al., 1995; Claria et al., 1996; Van Dyke and Serhan, 2003).

In addition to the omega-6 derived pro-resolving molecules, resolvins, and protectins are derived from the omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA; Van Dyke, 2007; Serhan and Chiang, 2008). They are able to stimulate anti-inflammatory and pro-resolving pathways similar to the lipoxins, but their binding sites on inflammatory cells differ from each other (Serhan et al., 2004; Van Dyke, 2007; Serhan and Chiang, 2008). Resolvins stimulate the resolution of inflammation through multiple mechanisms, including preventing neutrophil penetration, phagocytosing apoptotic neutrophils to clear the lesion, and enhancing clearance of inflammation within the lesion to promote tissue regeneration (Bannenberg et al., 2005; Hasturk et al., 2007; Schwab et al., 2007). The classic inflammatory eicosanoids (i.e., prostaglandins and leukotrienes), in addition to activating and amplifying the cardinal signs of inflammation, are also responsible for inducing the production of mediators that have both anti-inflammatory and pro-resolution activities, such as the lipoxins, resolvins, and protectins, reinforcing the active nature of the resolution process (Serhan et al., 2008). In humans, the aspirin-tolerant subjects generated both LXA4 and ATL, but aspirin-intolerant patients proved to have a diminished capacity to generate ATL and LX upon aspirin challenge (Sanak et al., 2000). In an experimental periodontitis rabbit model, animals are protected by LXA4 and transgenic (TG) rabbits over expressing 15 LO generate enhanced levels of LX, exhibit a reduced inflammatory phenotype, and are protected from bone loss in periodontal disease (Serhan et al., 2003). The treatment with Resolvin-E1 (RvE1) prevented and completely eliminated the signs of inflammation. In the RvE1 treated group, inflammation was completely eliminated; pocket depth was returned to normal and soft tissues returned to healthy levels and appearance (Figure 2). Regeneration of new cementum and bone with an organized periodontal ligament was observed (Hasturk et al., 2007). Restoration of crestal bone height, elimination of infrabony defects, and regeneration of new cementum, connective tissue, and bone with an organized periodontal ligament were signs of complete regeneration of tissues to pre-disease levels (Hasturk et al., 2007). Periodontitis was also shown to have a systemic impact elevating the levels of IL-1β and C-reactive protein (CRP) in all animals. Oral topical RvE1 therapy reduced systemic IL-1β and CRP levels. Rabbits treated with RvE1 showed an essentially complete recovery without any signs of local and
FIGURE 2 | Regulation of inflammation by resolvin-E1 in experimental periodontitis. (A) Periodontal disease was induced by ligature and Porphyromonas gingivalis application over 6 weeks in rabbits. Classical characteristic of periodontal disease including tissue and bone loss were observed. (B) Sites were treated either with RvE1 (1 mg/ml) or vehicle (ethanol) for an additional 6 weeks. RvE1 treatment did not only stop the disease progression but also reversed the tissue and bone loss and allowed the tissues to reach to a completely healthy state. Vehicle treatment did not have any impact on controlling the disease, conversely the disease continued to progress. (C) Histological evaluations confirmed the clinical observations where RvE1 treated sites showed no bone loss and no or minimal inflammatory cell activity. (D) Histomorphometric evaluations quantified the bone level changes during these treatments over 6 weeks. While RvE1 treatment resulted in bone gain, vehicle treatment showed worsening and lost more bone as a result of disease progression.

systemic inflammation (Hasturk et al., 2007) suggesting a complete return to tissue homeostasis. This mechanism is most likely regulated through an orchestrated series of events where in addition to neutrophils and lymphocytes, macrophages play the pivotal role.

MACROPHAGES AS A POSSIBLE LINK BETWEEN ORAL AND SYSTEMIC INFLAMMATION

The critical role of the macrophages in inflammatory diseases has been studied extensively in various organ systems in the human body. While the debate over the direction and cross-reactivity of local and systemic inflammation continues regarding which of the local specialized tissues are affected by systemic inflammatory changes and how the specific inflammatory processes in any part of the body have a generalized impact distant to the affected site, it is thought that the relationship is bidirectional (Offenbacher and Salvi, 1999; Amar et al., 2007; Ebersole et al., 2010; Hajishengallis, 2010). Such a dynamic response requires an intricate network of cellular and non-cellular components where macrophages are at the epicenter due to their extensive functional interactions with other cells and processes of inflammation. To this end, research has provided evidence of an oral and systemic connection in several diseases such as diabetes, cardiovascular diseases, and pathological conditions such as pre-term birth (Paquette et al., 1999; Nassar et al., 2007; Offenbacher et al., 2009).

We have previously studied macrophages and their role in the aggravation of inflammation in diabetics and identified critical markers of regulation at cellular signal transduction. These series of studies have demonstrated that oxidative stress plays a substantial role in the pathogenesis of diabetic complications. Superoxide anion is the first molecule generated during the respiratory burst of phagocytes, including macrophages, by NADPH oxidase. Either at rest or after stimulation with PMA or opsonized zymosan (OPZ), monocytes from people with diabetes produced significantly more anion than those from healthy individuals. The increased anion generation was found to be correlated with glyemic control (HbA1c) of patients. To clarify the impact of hyperglycemia on superoxide generation, normal human monocytes were then treated with receptor for advanced glycation end products (RAGE) ligands (advanced glycation end product, AGE protein and S100B) or high glucose media before stimulation. Both RAGE ligands and high glucose concentration increased...
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**FIGURE 3** | Local periodontal inflammation as a modifier of atherosclerotic changes in aortas of high cholesterol-fed rabbits.

(A) Atherosclerosis was induced by high cholesterol diet (0.5%) in rabbits over 13 weeks. Simultaneously, periodontal disease was also induced as explained above over a 6-weeks period. At 13 weeks, the aortas dissected en face and stained with Sudan IV for detection of lipid depositions. As a result of high cholesterol diet, rabbits developed early fatty streaks as indicated by Sudan IV stained lipid depositions mainly limited at the aortic arch and thoracic aorta. Rabbits challenged with *P. gingivalis* showed dramatically more and extended level of lipid depositions covering almost entire surfaces of thoracic and abdominal aortas. (B) Quantification of lipid covered area clearly showed that local periodontal inflammation significantly increases the atherosclerotic changes induced by cholesterol diet. (C) Periodontal disease was also more dramatic in those rabbits received high cholesterol diet suggesting a reciprocal relationship between local and systemic inflammations. (D) The severity of bone loss was positively correlated with degree of the fatty streaks (lipid depositions; \( r^2 = 0.9501 \)).

Anion generation from human macrophages. Notably, high glucose was associated with correspondingly increased osmotic pressure. This study demonstrated that RAGE ligands can significantly contribute to the hyper-responsive phenotype of diabetic monocytes and macrophages, which might be reversible by blocking RAGE or reducing RAGE ligands by controlling hyperglycemia (Ding et al., 2007a).

Hyperglycemic episodes in diabetes are closely associated with increased oxidative and nitrosative stress, which can trigger the development of diabetic complications. Hyperglycemia stimulates the production of advanced glycosylated end products, activates protein kinase C, and enhances the polyol pathway leading to increased superoxide anion formation. Superoxide anion interacts with nitric oxide, forming the potent cytotoxin peroxynitrite, which attacks various biomolecules in the vascular endothelium, vascular smooth muscle, and myocardium, leading to cardiovascular dysfunction (Pacher et al., 2005). High concentrations of hydrogen peroxide activate insulin signaling and induce typical metabolic actions of Czech et al. (1974). The pathogenetic role of nitrosative stress and peroxynitrite, and downstream mechanisms including poly(ADP-ribose) polymerase (PARP) activation. PARP activation can also up-regulate various pro-inflammatory pathways which leads to pathological modifications in adhesion molecule expression, angiogenesis, and other processes (Virag and Szabo, 2002).

In order to identify the specific signaling pathway through which the RAGE-mediated functional changes are effected in macrophages in diabetic people, we focused on the enzyme systems, which regulate the oxidative burst in macrophages. To this end, our research has shown that an alteration in the protein kinase C (PKC) family of intracellular enzymes, which plays a crucial role in signaling for a variety of cellular responses of mononuclear phagocytes including phagocytosis, oxidative burst, and secretion, are directly involved in the pathogenesis of the complications of diabetes. The consequences of PKC activation were evaluated by endogenous phosphorylation of PKC substrates with a phospho-specific PKC substrate antibody [pPKC(s)]. Phosphorylation of a 40-kDa protein was significantly increased in mononuclear phagocytes from diabetics as a downstream marker of PKC activation, and its phosphorylated form was found to be associated with the membrane. Through a wide range of techniques including the mass spectrometry, immunoprecipitation, and immunoblotting, we have identified this protein as pleckstrin. Phosphorylation and translocation of pleckstrin in response to the activation of RAGE suggested that pleckstrin was involved in RAGE signaling and AGE-elicited macrophage dysfunction. Suppression of
FIGURE 4 | Lipoxin A4, a resolution phase agonist, conferred similar actions with RvE1 on periodontal tissues challenged by *P. gingivalis* and ligature. (A) Periodontal inflammation was induced in transgenic and non-transgenic rabbits as described elsewhere for 6 weeks. Simultaneously, topical LXA4 (5–6 μg/site) was applied to the ligated sites in some non-transgenic animals. At 6 weeks, similar to RvE1, Lipoxin A4 resulted in significant reduction of tissue inflammation as a result of disease initiation. 15 LO overexpressing transgenic rabbits (15 LO-TG) exhibited no inflammation or tissue destruction and were completely protected from periodontal inflammatory changes. (B) The defleshed specimens clearly showed the amount of bone loss as a result of the periodontal disease induced by the human oral microorganism, *P. gingivalis* (left panel). LXA4 was capable of preventing these inflammatory changes and bone loss (middle panel), while the 15 LO-TG rabbits were not affected by disease induction, and were completely resistant to the disease (right panel). (C) Histological evaluations have confirmed the clinical observations and once again showed a complete protection in 15 LO-TG rabbits from inflammatory changes demonstrated by an unaffected healthy bony architecture (right panel). Topical LXA4 application protected from the destructive effects of periodontal disease as indicated by histological evaluations (middle panel).

pleckstrin expression with RNAi silencing revealed that phosphor- ylation of pleckstrin is an important intermediate in the secretion and activation pathways of pro-inflammatory cytokines (TNF-α and IL-1β) induced by RAGE activation. Thus, phosphorylation of pleckstrin up-regulated in diabetic mononuclear phagocytes was in part due to the activation of PKC through RAGE binding, and pleckstrin was a critical molecule for pro-inflammatory cytokine secretion in response to elevated AGE in diabetes in macrophages (Ding et al., 2007b).

Cardiovascular diseases and oral inflammation is also linked through the pivotal role of the macrophages. Epidemiological and recent clinical studies have implicated periodontitis as a risk factor for cardiovascular disease. Leukocytes can affect the vascular endothelial lining and can cause oxidation of low-density lipoprotein (LDL). Monocytes are induced to become macrophages, which take up modified lipoproteins and become lipid-laden “foam cells” (Paigen et al., 1987a,b). The local inflammation is sustained by secreting chemical mediators. Activated macrophages in the atherogenic plaque produce inflammatory cytokines (interferon, interleukin-1, and TNF-α), which induce the production of substantial amounts of interleukin-6. These cytokines are also produced in various tissues in response to infection and in the adipose tissue of patients with metabolic syndrome (Hansson, 2005). Interleukin-6, in turn, stimulates the production of large amounts of acute phase reactants, including CRP, serum amyloid A, and fibrinogen, by the liver (Ridker et al., 2000). CRP, the well-accepted marker of atherosclerotic disease, is shown to activate complement and accounts for LDL uptake by macrophages (Zwaka et al., 2001). The atherosclerotic lesion begins to bulge within the luminal wall and as the lesion progresses; the ECM is degraded by proteolytic enzymes and becomes susceptible to rupture. Thromboses can occur, occluding blood flow to the heart, which may eventually lead to infarction.

While macrophages play a central role in the development of atherosclerosis, specifically in the initial accumulation of cholesterol in the arterial wall (Ross, 1993), it has been suggested that infection and chronic inflammatory conditions such as periodontitis may influence the atherosclerosis process (Haraszthy et al., 2000). *P. gingivalis*, one of the major pathogens involved in periodontitis, has been detected in human atheromas (Deshpande...
suggesting that *P. gingivalis* infection may be associated with atherosclerosis. It has been proposed that bacteria or viruses may infect atherosclerotic plaques contributing to the inflammatory process. Distant infections may increase systemic inflammation through the release of toxins (i.e., bacterial LPS) or the leakage of chemical mediators into the circulation (Qi et al., 2003). Although multiple cross-sectional studies have supported these hypotheses by demonstrating a higher incidence of atherosclerotic complications in patients with periodontal disease (Mattila et al., 1989; Arbes et al., 1999) and suggest a strong link between periodontal inflammation and atherosclerosis (DeStefano et al., 1993), these observational studies are far from proving causation as proposed. Experimental animal models where periodontitis and atherosclerosis were developed in the same animal have been recently used to address this challenge. The ApoE-null mouse periodontal disease model was able to demonstrate that experimental induction of periodontal disease by serial inoculations of *P. gingivalis* exacerbated early atherosclerotic lesions (fatty streaks) within 4 months (Lalla et al., 2003). In addition, serum IL-6, aortic VCAM-1, and tissue factor antigen levels were increased in mice with *P. gingivalis* infection. In parallel, our group has shown that *P. gingivalis* induced periodontitis in rabbits dramatically increased lipid deposition in the aortas of cholesterol-fed rabbits compared to high cholesterol diet alone within 13 weeks (Jain et al., 2003). Animals with experimentally induced periodontitis had more extensive accumulations of lipids in the aorta compared to non-periodontitis animals (*P < 0.05*), and there was a positive correlation between the severity of periodontal disease and the extent of lipid deposition (*r*² = 0.9501; Figure 3). In this study, *P. gingivalis* 16S ribosomal RNA were not found in atheromatous plaques supporting the concept that rather than the bacteria itself, *P. gingivalis* cells or its vesicles released from periodontal lesions into the circulation may deliver virulence factor(s) such as LPS to the arterial wall to initiate or promote foam cell formation by macrophages and contribute to atheroma development (Qi et al., 2003). In a subsequent study, transgenic rabbits over-expressing 15-lipoxygenases and their response to inflammatory challenge were examined. Periodontal disease was initiated by topical *P. gingivalis* application. 15 LO-TG rabbits exhibited markedly reduced bone loss and local inflammation compared to non-transgenic rabbits where a significant amount of tissue destruction was observed (Figure 4). Further, application of topical aspirin-triggered lipoxin (LXA4) to the gingival site dampened the PMN-mediated tissue breakdown and bone loss suggesting that regulation of inflammation can provide an enhanced anti-inflammation status, which results in prevention of periodontal inflammation (Serhan et al., 2003). Overexpression of 15-lipoxygenase type I in transgenic rabbits increases the levels of endogenous lipoxin A4, which leads to prevention of periodontal inflammation as well as reduction of accelerated inflammatory events that contribute to atherosclerotic changes (Shen et al., 1996; Serhan et al., 2008).

**CONCLUSION**

Oral inflammatory processes involve microbial etiologic factors induce a series of host responses that mediate an inflammatory cascade of events in an attempt to protect and/or heal the tissues. It is becoming clear that the phenotype of the macrophage is central to determining the fate of the lesion; resolving; or chronic. Since the response of the macrophage is essential to health and disease, it is important to achieve a more complete understanding of the molecular events in this complex system. It is now becoming apparent that innate immune system cells are the determinants of the fate of the tissues and organs and are more than just transient, and their role is not limited to engulfing the invading microbes. Neutrophils and monocyte/macrophages are the key cells of the host response where their role go beyond the “defense” and is involved in the entire armamentarium of tissue homeostasis where protection, healing-repair, and regeneration are encoded.

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